Lumbar Discitis Caused by *Clostridium perfringens*

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We report here a rare case of chronic lumbar discitis caused by *Clostridium perfringens* in an elderly patient that was treated with a combination of β-lactams and clindamycin. Molecular analysis performed on the strain revealed an unusual toxin gene pattern.

CASE REPORT

An 83-year-old woman visited the emergency room for a worsening of her chronic lumbar back pain over the previous 6 months. Noteworthy medical history consisted of non-insulin-dependent diabetes, atrial fibrillation with a pacemaker fitted in, and cardiac insufficiency. Physical examination revealed left-side low back pain with lumbar disk 5 (L5) sciatica without any neurological deficit. Her body temperature was normal. Laboratory investigations revealed inflammation markers such as elevated C-reactive protein (80 mg/liter), leukocytosis (15×10^9/liter), and normocytic anemia (hemoglobin,10.5 g/dl; mean corpuscular volume, 90 μm^3). Procalcitonin was slightly elevated, at 0.11 μg/liter. Blood and urine cultures were negative. A computed tomography (CT) scan of the thoracolumbar spine showed a wide zone of bone erosions of the lower L4 and upper L5 endplates associated with a large vacuum phenomenon within the L4-L5 disk that correlated with multiple gas bubbles and fluid collection extending to the left paraspinal soft tissues and left proximate psoas attachment (Fig. 1). Magnetic resonance imaging (MRI) was not an option, since the patient was fitted with a traditional pacemaker for atrial fibrillation. As infectious discitis was suspected and blood cultures were negative, a CT guided-needle sample of the L4-L5 disk fluid collection was obtained and sent for analysis. The sample was inoculated into aerobic and anaerobic blood culture bottles. Cultures were positive in 72 h in only the anaerobic bottle. Gram staining showed large and sporulating Gram-positive rod-shaped bacilli. Subculture performed on blood agar plates under anaerobic conditions at 37°C showed growth in 24 h with numerous large colonies surrounded by a single zone of hemolysis. Matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) mass spectrometry (MicroFlex LT; Bruker Daltonics) performed directly on the colonies identified *Clostridium perfringens* (log score value of 2.39 matching *Clostridium perfringens* reference strain RV_BA_03_D LBK; MALDI Biotyper v2.3). The aerobic cultures remained negative after 48 h. Antimicrobial susceptibility testing was performed on our isolated strain using Etest strips (bioMérieux) and Clinical and Laboratory Standards Institute interpretative standards for anaerobes (1) after 48 h of incubation under anaerobic conditions (brucella agar supplemented with hemin at 5 μg/ml and vitamin K1 at 10 μg/ml). The strain was highly susceptible to all β-lactams, clindamycin, metronidazole, moxifloxacin, and rifampin. The strain was resistant to trimethoprim-sulfamethoxazole (Table 1). During the first 2 days, antibiotic treatment consisting of amoxicillin and clavulanic acid was given intravenously at a dosage of 2 g three times a day (t.i.d.). Then, intravenous bitherapy that was active against the isolated strain was initiated; this therapy consisted of 2 g of amoxicillin four times a day and clindamycin at 600 mg t.i.d. for 2 weeks, followed by oral monotherapy with clindamycin at 600 mg t.i.d. for 4 more weeks. The total duration of antibiotic treatment was 6 weeks, as recommended by French guidelines for bacterial discitis (2). The pain abated, and laboratory tests for inflammation returned to normal at the end of the treatment. Repeat imaging at 6 months yielded only a residual heterogeneous aspect of both vertebral plates without osteolysis; all these signs were considered sequelae of the infectious phenomenon. This outcome is consistent with other favorable outcomes reported in patients for whom antibiotic treatment included at least one β-lactam (3–6).

Clostridium perfringens is an anaerobic spore-forming Gram-positive bacillus that is ubiquitous in the telluric environment. It is also considered part of the normal intestinal flora. The virulence of *C. perfringens* is due mainly to toxin production and is involved in a number of human diseases, such as gas gangrene, food poisoning, infant necrotizing enterocolitis, and enteritis necroticans (7).

However, discitis due to *C. perfringens* is considered uncommon. To date, only five cases have been reported. In two patients, the discitis was iatrogenic, occurring promptly after spine surgery, suggesting transcutaneous inoculation of *C. perfringens* (3, 8). In three patients, clostridial discitis was considered a secondary bone infection from a primary gastrointestinal source that spread to the bloodstream (4–6). In our case, paraclinical investigations including full-body CT scans did not detect any digestive pathology and blood cultures remained negative after prolonged incubation. Colonoscopy was not an option because of heart failure during the hospitalization period. Nevertheless, translocation of *C. perfrin-*
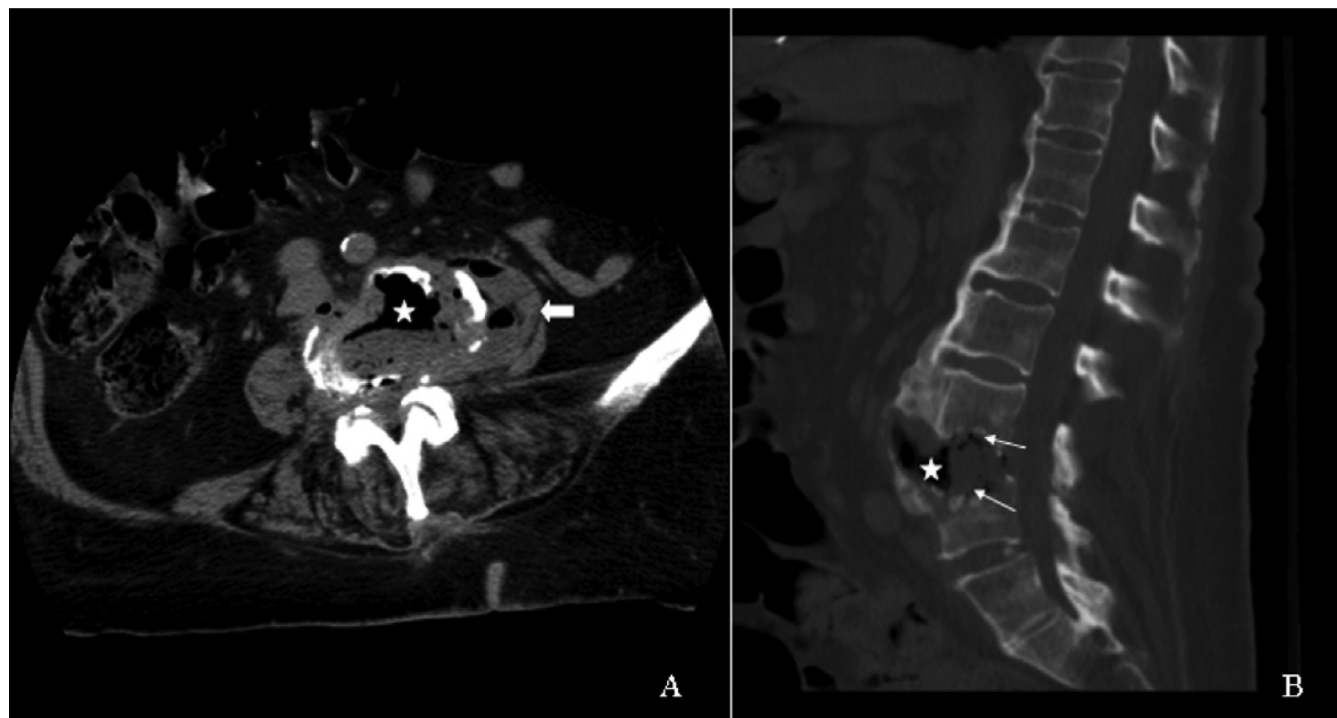
FIG 1 Computed tomography images of an axial section of a soft-tissue window on the L4-L5 disk (A) and a sagittal section of a bone window (B) are shown. A large central gas bubble (white star), erosions of the endplates (thin arrows), and fluid collection with a gas bubble in the left paraspinal soft tissues (wide arrow) are indicated.

gens from the intestinal tract with a bacteremia spreading to the lumbar spine could not be excluded.

The clinical presentation also suggested a well-tolerated chronic discitis process (low onset of pain and no signs of sepsis or acute toxic syndrome), which is unusual, since *C. perfringens* is considered a virulent pathogen responsible for severe infectious diseases, such as gas gangrene and necrotizing enterocolitis (7). In order to analyze its virulence factors, the isolated strain (CP54.2014) was sent to a reference laboratory (Centre National de Référence des Bactéries Anaérobies et du Botulisme, Pasteur Institute, Paris, France). PCR assays targeting genes encoding 10 major *C. perfringens* toxins were positive for only the alpha-toxin gene, which is highly unusual (see Table 2 for the list of toxin genes screened by PCR and references for the primers [9]). Interestingly, the phenotypical aspect of the hemolysis observed in our strain,

the display of a single zone of hemolysis, was consistent with these results (production of alpha-toxin alone). Usually, *C. perfringens* strains produce both alpha-toxin and theta-toxin (or perfringolysin O [PFO], encoded by the *pfoA* gene). However, some *C. perfringens* strains are defective in PFO production. For example, enterotoxigenic *C. perfringens* strains (e.g., strain SM101), which are responsible for food intoxication, contain the enterotoxin gene located on a large plasmid and a mutated *pfoA* gene (10, 11). The *C. perfringens* isolate CP54.2014 showed an unusual toxin gene pattern, including the absence of both *C. perfringens* enterotoxin (*cpe*) and *pfoA* genes. Indeed, PCR detection with internal primers for *cpe* and *pfoA* (Table 2) was negative. The presence of *pfoA* was further investigated with primers on the flanking regions of *pfoA*, one located in the upstream *pfoR* gene and the second in the downstream gene of *pfoA*, as previously described by Deguchi et al. (10). This PCR amplifies a 2,375-bp DNA fragment when *pfoA* gene is present and a 280-bp product when *pfoA* gene is deleted (10). Isolate CP54.2014 yielded no detectable PCR amplification product, which suggests an unusual sequence rearrangement/deletion in the *pfoA* gene region. These data are not surprising since *C. perfringens* is known to have a wide plasticity in its genome (12). However, only the whole-genome sequencing of this strain will accurately assess a genomic rearrangement and/or loss of toxin genes. Alpha-toxin production in CP54.2014 and the reference strain SM101 was monitored using an overnight culture supernatant in Trypticase-yeast extract medium and a *p*-nitrophenylphosphorylcholine assay as described by Jepson et al. (13). This assay confirmed that CP54.2014 produced alpha-toxin (data not shown).

In conclusion, this report is interesting from a medical and

TABLE 1 Antimicrobial susceptibilities of *C. perfringens* determined with the Etest method and clinical categorization using CLSI breakpoints

Antimicrobial agent	MIC (μg/ml)	Clinical categorization[a]
Amoxicillin	0.032	S
Amoxicillin-clavulanic acid	0.047	S
Piperacillin-tazobactam	0.75	S
Metronidazole	1.5	S
Clindamycin	0.047	S
Moxifloxacin	0.019	S
Trimethoprim-sulfamethoxazole	32	R
Rifampin	0.002	S

[a] S, susceptible; R, resistant.

TABLE 2 List of toxin genes screened by specific PCR and reference for primers used in the study

C. perfringens toxin	Toxin gene	Primer name	Primer sequence (5'→3')	Positions (nt)[a]	Size (bp)	Accession no.	Reference or source
Alpha-toxin	*plc*	PL3	AAGTTACCTTTGCTGCATAATCCC	1676–1699R	236	AF204209	Popoff et al., 2003 (9)
		PL7	ATAGATACTCCATATCATCCTGCT	1418–1440			
C. perfringens enterotoxin	*cpe*	P145	GAAAGATCTGTATCTACAACTGCTGGTCC	643–674	425	M98037	Popoff et al., 2003 (9)
		P146	GCTGGCTAAGATTCTATATTTTTGTCCAGT	1039–1068R			
Beta-toxin	*cpb1*	P1677	TCAATTGAAAGCGAATATGCTG	861–882	621	L13198	This work
		P1678	CTATGGACGCTCCCCCTATT	1481–1462			
Beta2-toxin	*cpb2*	P465	TTTTCTATATATAATCTTATTTGTCTAGCA	978–948R	277	L77965	Popoff et al., 2003 (9)
		P466	AGTTTGTACATGGGATGATGAACTAGCACA	723–752			
Epsilon-toxin	*etx*	P497	GTCCCTTCACAAGATATACTAGTACC	1051–1101	172	M80837	Popoff et al., 2003 (9)
		P498	CCTAGGAAAAGCTAAATAACTAGG	1183–1223R			
Iota-toxin, Ia component	Ia gene	P499	TAATTTTAACTAGTTCATTTCCTAGTTA	1506–1546	317	X73562	This work
		P500	TTTTTGTATTCTTTTTCTCTAGGATT	1783–1823R			
Iota-toxin, Ib component	Ib gene	P501	CTTATGAAAAAAATGGCTATACTA	3545–3585	324	X73562	This work
		P502	GTTTTACTATTTGTAGTAGCCCTAGAAA	3829–3869R			
Delta-toxin	*cpd*	P934	CTAAATGCAAATTATGCTGTT	671–691	400	EU652406	This work
		P935	TGTTTCTTCAATTTTACTATCTGG	1075–1032			
Theta-toxin	*pfo*	P1685	TCCATCAGATCTTTTTGATGACA	720–742	495	BA000016	This work
		P1686	TGTGCAACATAGGCTCCACTAT	1214–1193			
TpeL	*tpeL*	P1557	ATATAGAGTCAAGCAGTGGAG	2810–2830	464	AB262081	This work
		P1558	GGAATACCACTTGATATACCT	3255–3275			
NetB	*netB*	P1644	TTTGTTGAGACTAAGGACGGTT	627–648	266	EU143239	This work
		P1645	TCGCCATTGAGTAGTTTCCC	893–874			

[a] R, reverse primer.

therapeutic point of view. The progressive clinical presentation in our patient, with no evidence of septic or toxic shock syndrome associated with a *C. perfringens* strain displaying an unusual gene pattern, is uncommon. Hopefully, the spread of new genomic tools such as whole-genome sequencing will help detect unusual genomic rearrangement in *C. perfringens* strains and determine if some genomic profiles correlate with specific clinical patterns in human pathogenesis. To date, discitis caused by anaerobic bacteria remain rare clinical entities, and guidelines concerning the type of antimicrobial therapies have yet to be published. The favorable outcomes involving β-lactams and clindamycin in this case, as assessed by previous reports, point out the efficiency of these two classes of antibiotics in the treatment of discitis due to anaerobic bacteria.

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